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Donor and recipient treatment with the Lazaroid U-74006F do not

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Abstract

Objective: U-74006F is the only Lazaroid which is currently in clinical use. A number of experimental studies demonstrate that Lazaroids reduce ischemia/reperfusion injury in various organ systems. We evaluated the effect of U-74006F on reperfusion injury in a large animal model of lung allo-transplantation. **Methods**: Two different treatment modalities were evaluated and compared with corresponding control groups. Unilateral left lung transplantation was performed in 21 weight-matched pigs (24–31 kg). Donor lungs were flushed with 1.5 l cold (1°C) LPD solution and preserved for 20 h. In group I (n = 5), donor animals were pretreated with U-74006F (10 mg/kg i.v.) 20 min before harvest. In addition U-74006F was added to the flush solution (10 mg/l). In group III (n = 6), the Lazaroid was given to the donor before flush and to the recipient before reperfusion (3 mg/kg i.v.). Group II and IV (n = 5) served as control. One hour after reperfusion, the recipient contralateral right pulmonary artery and bronchus were ligated to assess graft function only. Extravascular lung water index (EVLWI), mean pulmonary artery pressure, cardiac output, and gas exchange were assessed during a 5 h observation period. Lipid peroxidation (TBARS) and neutrophil migration (MPO activity) were measured at the end of the assessment in lung allograft tissue. **Results**: A significant change of TBARS concentration was shown in group III (group III 78.7 ± 4.6 pmol/g vs. group IV 120.8 ± 7.2 pmol/g (P = 0.0065) normal lung tissue 41.3 ± 4.2 pmol/g). MPO activity was reduced in group III 3.74 ± 0.25 ΔOD/mg per min vs. group IV 4.97 ± 0.26 ΔOD/mg per min (P = 0.027), normal lung tissue 1.04 ± 0.27 ΔOD/mg per min). Pulmonary hemodynamics and gas exchange after reperfusion did not differ between groups. In group I and III, a tendency towards a reduced EVLWI was noted. **Conclusion**: We conclude that combined treatment of donor and recipient with U-74006F reduces free radical mediated injury in the allograft. However, this intervention did not result in a significant reduction of post-transplant lung edema or improvement of pulmonary hemodynamics. Donor pretreatment alone did not improve lung allograft reperfusion injury. These results indicate that the benefit of U-74006F is too small to consider clinical application in lung transplantation.

Keywords: Lung transplantation; Reperfusion injury; Lazaroids; Animal model; Swine

1. Introduction

During the last 10 years, lung transplantation has become an accepted treatment modality for patients with end stage lung disease. Despite improved organ preservation and perioperative management, graft dysfunction remains a significant and unpredictable clinical problem and occurs in a severe form in over 10% of the recipients.

Activation of humoral and cellular responses are thought to be crucial for the development of post-transplant reperfusion injury. Important mediators include neutrophil granulocytes, the complement system and oxygen free radicals.

Lazaroids are a group of 21-aminosteroids, which were originally synthesized as inhibitors of lipid peroxidation after trauma, bleeding, or ischemia of the CNS. They reduce lipid peroxidation [1,2] and subsequent edema formation

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[3,4] in combination with minimal glucocorticoid- or immunosuppressive side effects [5]. The protective capacity of these compounds made them candidates for use in other systems, e.g. post-transplant ischemia/reperfusion. A number of in vivo and ex vivo experiments of ischemia/reperfusion injury using different Lazaroid compounds were published with contradictory results [6,7].

However, because of unfavorable pharmacodynamic and pharmacokinetic properties such as toxicity and instability of other Lazaroids [2], U-74006F is still the only substance of this group in clinical use. The purpose of this study was to evaluate the effect of U-74006F on post-transplant reperfusion injury using two different treatment modalities – donor pretreatment alone and combined donor/recipient treatment – in a large animal model of unilateral lung transplantation following prolonged preservation.

2. Materials and methods

2.1. Animals and operative procedure

Twenty-one weight-matched pairs of outbreed pigs served as donors and recipients. Harvest and left lung transplantation were performed as previously reported [8].

2.2. Study groups

In group I ($n = 5$) donor animals were pretreated with the 21-aminosteroid U-74006F (Freedox®, generously provided by Pharmacia Upjohn AG, Dübendorf, Switzerland) 20 min before flush in a dose of 10 mg/kg i.v. over 10 min. In addition U-74006F was added to the low potassium dextran (LPD) solution (Perfadex®, generously provided by Upjohn/Medisan Pharmaceuticals AB, Uppsala, Sweden) in a dose of 10 mg/l. In group III ($n = 6$), the Lazaroid was given to the donor before flush and to the recipient before reperfusion (3 mg/kg i.v.). Group II and IV ($n = 5$) served as control, no 21-aminosteroid was administered. The total preservation time was 20 h in all groups. In group I and II, the transplantation was performed after 18 h of cold ischemia (1°C) and a 2 h period of warm ischemia (20°C). In this first series of experiments, donor preparation and implantation were performed without topical cooling of the graft. In groups III and IV, the grafts were cooled topically with ice slush during dissection and implantation to prevent warm ischemia as in clinical practice. U-74006F was administered according to the manufacturers instructions based on toxicity and pharmacological characteristics.

All animals received humane care in compliance with the European Convention on Animal Care. The protocol was approved by the local animals study committee.

2.3. Assessment

One hour after reperfusion of the transplanted lung, the right pulmonary artery and the right main bronchus were ligated to assess allograft function only. During the assessment period anesthesia was maintained with Fluothane 1.5%. Systemic arterial, pulmonal arterial, central venous and left atrial pressure were recorded continuously. Arterial and mixed venous blood were collected for gas analysis every 60 min.

At the end of the assessment period, 5 h after reperfusion, the animals were sacrificed. Upper lobe allograft samples were submitted to histologic examination and tissue MPO and TBARS assay.

2.4. Extravascular lung water

Extravascular lung water as direct assessment of reperfusion edema was measured as previously described [8].

A fiberoptic catheter (System Cold Z-021, Pulsion, Munich, Germany) is advanced via the external carotid artery into the descending aorta. The indicator bolus consists of two components. Indocyanine green serves as intravascular marker and ice cold 5% glucose as a thermal intra- and extravascular indicator. The bolus is injected via the external jugular vein with a temperature controlled injector. The dilution curves for dye and temperature are recorded simultaneously in the descending aorta with the thermistor tipped fiberoptic catheter. Thoracic intravascular and extravascular fluid volumes are determined based on the measurement of the mean transit times for thermal and dye indicators and of the decay time volumes calculated from the indicator dilution curves as described previously [9]. The lung water computer (System Cold Z-021, Pulsion, Munich, Germany) determines the mean transit time for the thermal indicator and for the dye indicator and calculates total thermal volume (ITTV), intrathoracic blood volume (ITBV), and extravascular thermal volume (ETV) [10]. The extravascular thermal volume (ETV) is calculated as follows: ETV = ITTV − ITBV. All measurements were made hourly in triplicate. The mean value was used for analysis.

2.5. Myeloperoxidase assay

Donor and recipient lung samples were frozen immediately and stored at −70°C until assay. Quantitative MPO activity was determined as previously described [11]. Enzyme activity is expressed as the change in optical density unit per milligram of tissue protein per minute (ΔOD/mg per min).

2.6. TBARS assays

The thiobarbituric acid-reactive substance (TBARS) levels in lung tissue were measured according to the method of Ohkawa et al. [12] with 10% wet weight per volume homogenate. TBARS levels were determined by reference to a standard curve of 1,1,3,3-tetramethoxypropane (Sigma

Chemicals, Buchs, Switzerland), and the results were expressed as picomoles of malondialdehyde (MDA)/g of wet lung.

2.7. Statistical analysis

All values are given as the mean ± standard error of the mean (SEM). Donor weight, recipient weight, total preservation time, warm ischemic time, TBARS assay and MPO assay were checked for normal distribution within groups and analyzed by unpaired *t*-test. Gas exchange, hemodynamic parameters and extravascular lung water were assessed by repeated measures ANOVA and planned comparison was applied. Analysis was performed by ANOVA (StatView 4.5, Abacus Concepts, 1995). *P*-values less than 0.05 were considered significant.

3. Results

3.1. Characteristics of experimental groups

No statistical differences between groups (I vs. II and III vs. IV) were noted in donor weight, recipient weight, total preservation time and warm ischemic time (Table 1).

3.2. Reperfusion edema

At the end of the 5 h assessment period, only a tendency towards a reduced allograft reperfusion edema in comparison with the controls was noted (group I 5.2 ± 0.2 ml/kg vs. group II 6.7 ± 1.1 ml/kg, $P = 0.16$, and group III 7.4 ± 0.6 ml/kg vs. group IV 9.2 ± 0.9 ml/kg, $P = 0.13$) (Fig. 1).

3.3. Gas exchange

PaO_2 levels in the Lazaroid treated animals were higher at the end of the 5 h assessment period (group I 73.52 ± 2.30 kPa vs. group II 69.05 ± 1.61 kPa and group III 74.8 ± 2.1 kPa vs. group IV 61.7 ± 7.6 kPa), but statistical analysis did not demonstrate a significant difference between groups.

3.4. Hemodynamic parameters

No statistically significant difference between groups was noted in cardiac output (group I 2.5 ± 0.3 l/min vs. group II 3.1 ± 0.2 l/min and group III 3.6 ± 0.2 l/min vs. group IV 3.1 ± 0.4 l/min), pulmonary vascular resistance (group I 638 ± 41 dynes/s per cm^{-5} vs. group II 571 ± 62 dynes/s per cm^{-5} and group III 528 ± 82 dynes/s per cm^{-5} vs. group IV 580 ± 85 dynes/s per cm^{-5}) and pulmonary arterial pressure (group I 27 ± 1 mmHg vs. group II 30 ± 1 mmHg and group III 31 ± 1 mmHg vs. group IV 32 ± 3 mmHg) during the 5 h assessment period (Fig. 2).

3.5. PMN migration

Allograft MPO activity was reduced in group III animals in comparison with the corresponding control group (group I 3.78 ± 0.30 ΔOD/mg per min vs. group II 3.25 ± 0.37 ΔOD/mg per min and group III 3.74 ± 0.25 ΔOD/mg per min vs. group IV 4.97 ± 0.26 ΔOD/mg per min, ($P = 0.027$). MPO activity in normal unflushed lung tissue was 1.04 ± 0.27 ΔOD/mg per min (Fig. 3).

3.6. TBARS

The malondialdehyde concentration as expression for lipid peroxidation was significantly reduced in group III (group I 126.9 ± 15.9 pmol MDA/g vs. group II 120.8 ± 11.8 pmol MDA/g and group III 78.7 ± 4.6 pmol MDA/g vs. group IV 120.8 ± 7.2 pmol MDA/g, normal lung tissue 41.3 ± 4.2 pmol MDA/g) ($P = 0.0065$) (Fig. 3).

4. Discussion

A number of mechanisms of the non-specific immune system mediate lung allograft ischemia/reperfusion injury. This acute lung injury manifests as post-transplant lung edema with subsequent increase of pulmonary vascular resistance and reduced gas exchange. One of the most important mediators of ischemia/reperfusion injury are superoxide radicals, which are generated during preservation and reperfusion. Oxygen free radicals damage membranes and organelles by forming lipid peroxides which alter the configuration and function of these structures.

Lazaroid molecules act as membrane stabilizers by solving in the lipid bilayer of cell membranes with the hydrophobic steroid moiety located in the lipid phase and the

Table 1
Characteristics of experimental groups

Group (treatment)	I (Donor)	II (Control)	III (Donor + Recipient)	IV (Control)
Donor weight (kg)	26.2 ± 0.78	28.2 ± 1.27	27.2 ± 1.1	27.1 ± 1.2
Recipient weight (kg)	25.5 ± 0.55	27.8 ± 1.27	26.5 ± 0.6	27.6 ± 2.3
Total preservation time (min)	1221 ± 11	1215 ± 16	1180 ± 3	1218 ± 16
Warm ischemic time (min)	146 ± 7	148 ± 7	44 ± 5	46 ± 4

No statistical differences were noted in donor weight, recipient weight, total preservation time and warm ischemic time between the corresponding groups.

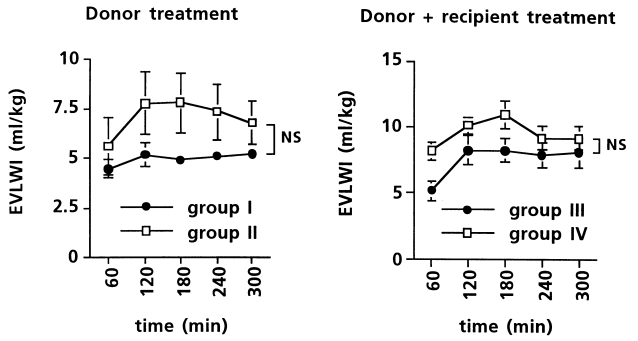

Fig. 1. Extravascular lung water index (EVLWI) in the pulmonary allograft in the control groups increased during the first 120 min after reperfusion followed by a slight decrease. Lazaroid treatment did not reduce EVLWI significantly (groups I and III).

more hydrophilic heterocyclic ring system near the phospholipid head groups [13]. In addition, Lazaroids are known to act as potent free radical scavengers [2].

In experimental and clinical studies, a beneficial effect of Lazaroids after neuro trauma has been demonstrated [1–4]. In the field of organ preservation, a considerable amount of work has been done to evaluate the effects of Lazaroids with contradictory results.

Salahudeen et al. noted that renal function improved with U-74006F treatment after transplantation in rats. This effect correlated with a decrease of plasma and tissue MDA levels [14]. In contrast, Ishizaki and Wang found no significant changes in lipid peroxidation with U-74006F, but they observed a clear decrease with a definite dose of U-74500A [6,13]. In a previous study reported by Killinger et al., different Lazaroid compounds were compared. U-74006F in contrast to U-74500A did not improve endothelial cell viability [7]. U-74500A was found to be a more potent inhibitor of iron-catalyzed lipid peroxidation than U-74006F, but this compound has not been further developed due to pharmaceutical instability and rapid elimination in vivo [2]. The two most important reasons for the decision to choose U-74006F for clinical application were the low toxicity and the best solubility in clinically applicable solvents (E. Hall, personal communication).

Lipid peroxides stimulate cytokine production and induce

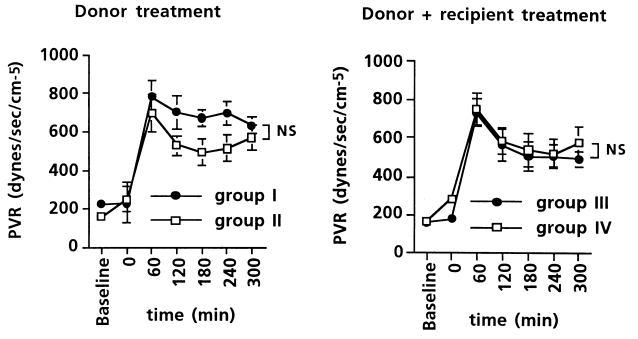

Fig. 2. Pulmonary vascular resistance (PVR) increased at the time of exclusion of the right native lung in all groups. Lazaroid treatment did not reduce this increase.

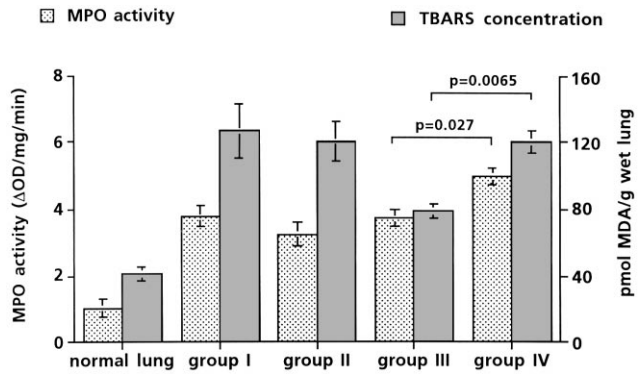

Fig. 3. PMN migration (MPO activity) and thiobarbituric acid reactive substance (TBARS) in lung allograft tissue at the end of the assessment. A significant reduction of both parameters in comparison with the controls could be demonstrated when the Lazaroid was given to the donor and the recipient (group III).

expression of adhesion with subsequent leucocyte activation and migration. Neutrophil activation highly correlates with the severity of lung injury [15], and Lazaroids are thought to reduce neutrophil migration after transplantation. Palma-Vargas reported a decrease in MDA levels and neutrophil infiltration with U-74389G [16], but U-74006F did not reliably reduce neutrophil tissue migration following ischemia/reperfusion in vivo [17] and in vitro [18,19]. In the experiments of Ishizaki, the neutrophil infiltration was even higher in the U-74006F group in comparison with U-74500A and U-74389G treated animals [6]. In the present study, we registered a significant decrease of neutrophil migration into the allograft after U-74006F administration in the group with combined treatment of donor and recipient (Fig. 3).

A number of authors previously evaluated the effect of Lazaroids in lung transplantations. Tanoue et al. noted a positive effect on pulmonary hemodynamics and gas exchange with U-74500A-treatment in a canine single left lung transplantation model [20]. Du et al. described similar results in a partially in vivo rat transplant model [21]. A favorable effect on gas exchange, however, could only be detected after 12 h of cold ischemic storage. After a 6-h preservation period, no difference between control and Lazaroid treated groups was found. Furthermore, the lung water content, which clearly raised after reperfusion, did not differ between groups. In our experiments, we could only demonstrate a tendency towards reduced lung edema with both treatment modalities, and no improvements in hemodynamic parameters nor gas exchange after Lazaroid treatment were noted.

Several ways of Lazaroid administration are described in the literature. It was added to the flush solution and/or to the recipient at the time or shortly before reperfusion. However, because the process of lipid peroxidation is thought to start at the onset of ischemia and is accelerated during reperfusion [22], we choose donor pretreatment before organ harvest as a reasonable way of administration in the first series of experiments. Sasaki et al. evaluated donor in vivo admin-

istration of Lazaroid U-74389G [23] and confirmed the results of Lambert and Egan, which demonstrate that a free radical scavenger must be present at the time of harvest to be effective [24]. In the study of Hausen et al., in a rat model of double-lung transplantation, donor treatment with U-74389G was more effective than administration to the recipient only [25]. The importance of treating the residual neutrophil pool in the donor lung as well as preventing lipid peroxidation caused by oxygen-free radicals released during ischemia from donor endothelial cells are thought to be important. Our results, however, indicate that donor treatment alone with U-74006F is not sufficient.

In preliminary studies, we noted that after 20 h of cold ischemic storage, donor treatment with U-74006F did not ameliorate reperfusion injury in this model [26]. As it is thought that oxygen free radicals are produced during warm ischemia, we extended the warm ischemic period in part one of the experiment (groups I and II) to 2 h before implantation.

An ischemic time of 20 h was chosen in this experiment to produce a severe reperfusion injury. Following this ischemic damage, the animals still tolerate ligation of the right pulmonary artery which allows to assess isolated graft function. If a longer preservation period is used, a rapid decrease of allograft gas exchange is noted and the animals die from right heart failure. It could be hypothesized that an ischemic time of 20 h is too long to demonstrate the protective effect of Lazaroids, however, other groups reported in a canine model improved outcome after liver and lung transplantation with even longer ischemic times [20,27].

We conclude that combined treatment of donor and recipient with U-74006F, which is still the only clinically used Lazaroid, reduces free radical mediated injury in the allograft, however, this intervention did not improve neither post-transplant lung edema nor pulmonary hemodynamics. Donor pretreatment alone did not improve lung allograft reperfusion injury. These results indicate that the benefit of Lazaroids is too small to consider clinical application in lung transplantation.

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